

## Rate Maintenance of Cell Division in *Escherichia coli* B/r: Analysis of a Simple Nutritional Shift-Down

ARIEH ZARITSKY<sup>1</sup> AND CHARLES E. HELMSTETTER<sup>2\*</sup>

*Department of Biological Sciences (Cell Biology), Florida Institute of Technology, 150 West University Boulevard, Melbourne, Florida 32901,<sup>2</sup> and Department of Life Sciences, Ben-Gurion University of the Negev, Be'er-Sheva 84105, Israel<sup>1</sup>*

Received 15 June 1992/Accepted 2 October 1992

**A competitive (nonmetabolizable) inhibitor of glucose uptake,  $\alpha$ -methylglucoside, was used to limit the growth of *Escherichia coli*. Cell division during such a nutritional shift-down was studied in batch cultures and with the "baby-machine" technique. Following a brief delay, the rate of division was maintained for 60 to 70 min in batch cultures and for an extended period in the baby machine. Decreases in cell size were due, in part, to a possible reduction in the mass per chromosome origin at the time of replication initiation and a shorter time interval between initiation and the subsequent division. These unusual findings suggest that this method for abrupt change in growth rate without modifying repression patterns is useful for studying the control of various aspects of the bacterial cell.**

A bacterial cell divides  $C + D$  min after initiation of chromosome replication (10). Initiation capacity builds up during its preceding doubling time,  $\tau$  min. Cell mass at initiation is, to a first approximation,  $2^n$ -multiple of a minimal value,  $M_i$ , where  $n$  is an integer greater than or equal to  $(C + D)/\tau$  (5, 25). The resultant increase of cell size with growth rate,  $1/\tau$ , at least in *Escherichia coli* and *Salmonella typhimurium*, is associated with enlargement in both cell dimensions (length and diameter) (26, 29, 31) with very little change in shape (length/diameter ratio) (30). Mutants with an altered  $M_i$  have been isolated, and some biochemical steps have been deciphered (1, 2, 23, 28), but the mechanisms that govern and integrate these processes (initiation and termination of chromosome replication, cell division and shape determination, and chromosome segregation) in the living cell are still obscure (for example, see reference 4).

Much of our understanding of the physiology of a bacterial cell stems from the experimental procedure which perturbs the steady state of exponentially growing cultures in a well-controlled way, the nutritional shift-up (for example, see references 1 and 17-20; for a review, see reference 3). The observed dissociation between the rates of RNA, DNA and protein syntheses and of nucleoid and cell division during a transition from one steady state to another were crucial in establishing the relationship between chromosome replication and cell division (9-11) and for pursuing studies on the mechanism of ribosomal biogenesis and the activity of the protein-synthesizing system (for reviews, see references 14 and 19). This approach also pointed to a way for discovering the mechanism of cell shape determination by the intriguing observation of length overshoot before the shifted cells attain their new steady-state dimensions (7, 29).

In spite of the prevalent statement that the new, higher rate of culture mass growth is achieved immediately after the shift to richer media (for example, see reference 22), a better approximation clearly describes it as a relatively slow process (31). This is due to the large changes in the repression-induction patterns between two sets of genes required under

the different growth conditions employed in such an experiment (14). This slow approach to the new steady state blurs the picture when detailed studies on growth control are desired. Reaching the new steady state in shift-down experiments is even slower (for reviews, see references 3 and 20).

It was therefore comforting to find shift-down conditions with virtually no modifications in the pattern of promoter activities, by suddenly reducing the carbon and energy source availability without replacing it. This effect has been achieved both (i) in transport mutants (27) and (ii) by competitive inhibition of glucose uptake by its analog  $\alpha$ -methylglucoside ( $\alpha$ MG) (16). The latter method has been exhaustively exploited to study the control of protein and RNA syntheses (8, 15, 21) but has never been employed to study other aspects of bacterial physiology.

Here, we describe studies on the details of cell division following such an abrupt nutritional shift. Additional data which were obtained with the differential, backward method, the so-called "baby machine" (10), disclose some new information on the coordination between chromosome replication and cell division.

**Maintenance of cell division rate.** If not stated otherwise, *E. coli* B/r (F26; *thy his*) was grown at 37°C in minimal medium (9) which contained 0.1% glucose as the sole carbon source and which was supplemented with 10  $\mu$ g of thymine and 50  $\mu$ g of histidine per ml. Upon addition of  $\alpha$ MG to steadily growing cultures (Fig. 1), mass growth rate was immediately reduced and then recovered somewhat to reach a new steady-state level, as was seen previously (8). Cells continued to divide at approximately the preshift rate (1.45  $h^{-1}$ ) for about 65 min, at which time they slowed abruptly to the new steady-state level (0.75 doublings per h). Cell sizes decreased during this interval because mass increased more slowly than cell number. Maintenance of the rate of cell division was evident when the ratio of the analog concentration to that of glucose was 10 or lower (data not shown).

**Baby-machine (membrane elution) experiments.** To substantiate this result, the baby-machine technique was exploited. This method (9) measures division rates directly rather than measuring cumulative cell numbers, and thus it is a differential method. A steadily growing culture in minimal medium with 0.1% glucose was mounted on a filter and

\* Corresponding author. Electronic mail address: helmstetter@roo.fit.edu.

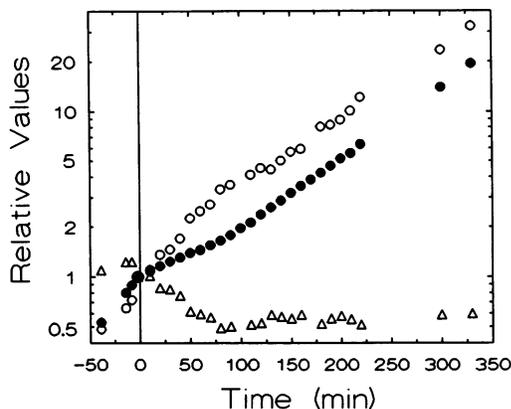


FIG. 1. Relative values of dry mass per ml (●), cell concentrations (○), and cell sizes (△) during a nutritional shift-down. Cells were counted with a Coulter electronic particle counter, and culture dry mass was determined with a Gilford N300 spectrophotometer at 450 nm ( $A_{450}$ ). Relative cell size was expressed by the ratio  $A_{450}/\text{cell}$ . All values were normalized to 1.0 at  $t = -2$  min. The culture was diluted into fresh, prewarmed identical medium at 102 min (1:2.7) and 205 min (1:7), to keep  $A_{450}$  at  $\leq 0.25$ .

eluted in the usual manner but with medium containing 1%  $\alpha$ MG as well ( $[\alpha\text{MG}]/[\text{glucose}] = 10$ ) (Fig. 2). Three major observations are noteworthy here. A slight initial reduction in division rate caused the subsequent divisions to be shifted by a phase of the same magnitude (about 15 to 20 min). Once formed, this phase was sustained for at least 4 generations, indicating that all cells in the original exponentially growing population responded similarly to  $\alpha$ MG, irrespective of age, by temporarily slowing the processes leading to division. This conclusion was supported by a series of similar experiments with various concentrations of  $\alpha$ MG (Fig. 3), in each of which the division delay was a function of the analog concentration.

Much more surprising is the long-term continued orderly divisions of the membrane-attached cells during elution with the analog, with little change in the rate of cell division. This differed from the observations obtained with batch cultures (Fig. 1) and may be explained by different conditions to which attached cells are exposed or by modified cell cycle parameters (or both). Membrane-attached cells are flushed

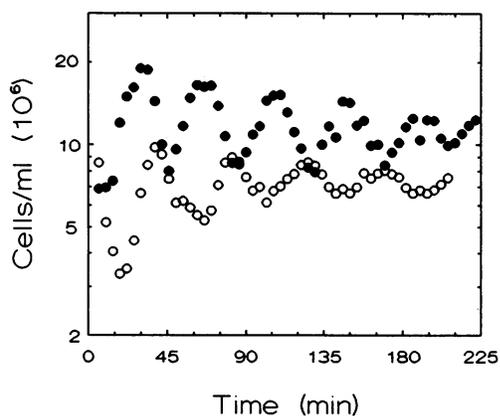


FIG. 2. Cell concentrations as a function of time in the eluates of control (●) and of  $\alpha$ MG-treated (○) baby-machine cultures.

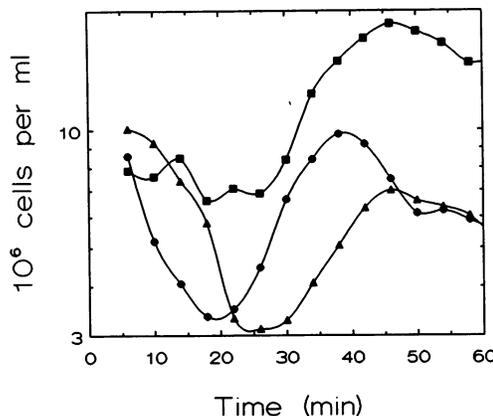


FIG. 3. Cell concentrations in the eluates of baby-machine cultures treated with 0.1% (●), 1.5% (▲), and 2% (■)  $\alpha$ MG.

continuously with fresh, oxygenated medium containing an invariant  $[\alpha\text{MG}]/[\text{glucose}]$  ratio, which is not the case in batch cultures. Furthermore,  $\alpha$ MG-6-phosphate, which is the intermediary metabolite accumulating in  $\alpha$ MG-exposed cells (14), may be flushed out of the membrane-attached cells constantly by the fresh elution medium. These possibilities may be related, directly or indirectly, but are indistinguishable on the basis of the present data.

In spite of the continued orderly divisions, cell size (whether volume [data not shown] or mass) decreased for 60 min, to achieve a new steady-state level (Fig. 4). This decrease in cell mass without concomitant significant decrease in division frequency is consistent with a reduction in one or more cell cycle parameters ( $C$ ,  $D$ ,  $M_i$ ) upon downshifting of the culture.

**Rates of division following pulse-shifting of cultures.** If addition of the analog induced such a change in the cell cycle, there should be a transient, abrupt increase in division rate approximately  $C + D$  min after its addition. As a dramatic example, if  $\alpha$ MG reduced  $M_i$  by one-half instantaneously, then all cells in the population would divide in a synchronous burst  $C + D$  min after its addition. However, this effect may be masked in batch culture by whatever

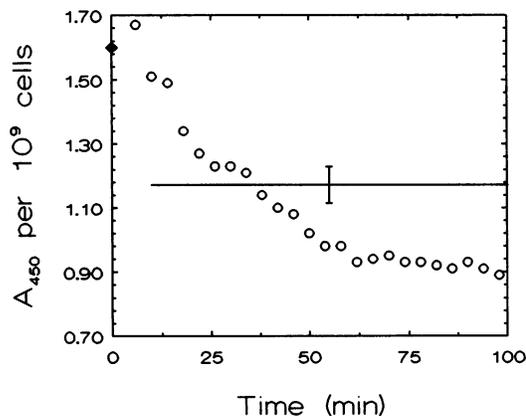


FIG. 4. Cell sizes in the eluate of a baby-machine culture treated with 1%  $\alpha$ MG (○). The horizontal line and bar indicate the mean cell size and its standard deviation, respectively, in the eluate of an untreated culture. ◆, mean cell size in the batch culture prior to membrane attachment.

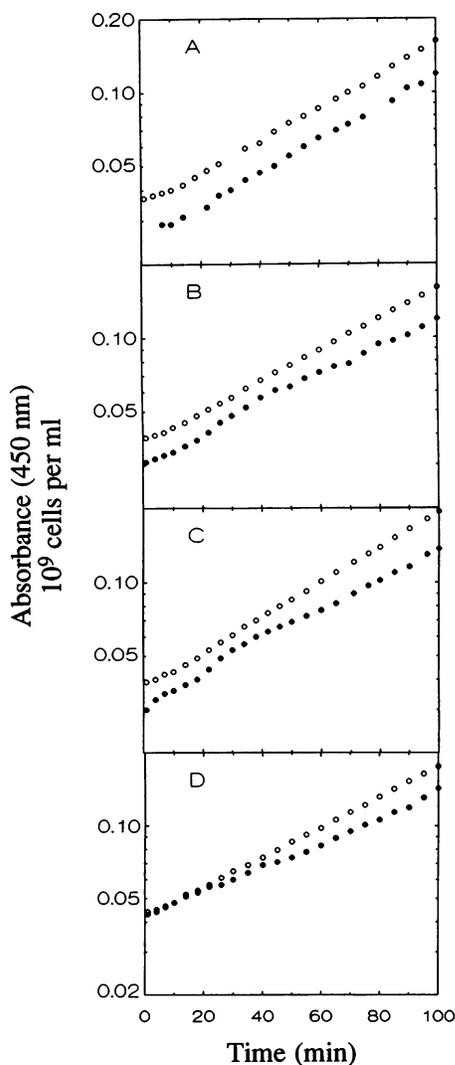


FIG. 5. Absorbance (○) and cell concentrations (●) in diluted cultures following pulse treatment with  $\alpha$ MG for 5 min (A), 20 min (B), 30 min (C), and 40 min (D).

causes growth of the cells to eventually slow down compared with their growth on the membrane filter. A short pulse may be sufficient to demonstrate the effects on cell cycle parameters before the adverse influence in batch culture is evident. Cultures were therefore pulsed with the analog for various lengths of time and then shifted back by a 10-fold dilution and addition of glucose to 1%, which drops the ratio  $[\alpha\text{MG}]/[\text{glucose}]$  by a factor of 100 (from 10 to 0.1) instantaneously without modifying the medium osmolarity. The rate of cell division did indeed increase slightly after  $C + D$  min when 20- and 30-min pulses were employed (Fig. 5B and C), supporting the hypothesis that  $C$ ,  $D$ , and/or  $M_i$  is reduced when the cells are exposed to  $\alpha$ MG. A 5-min exposure to the analog (Fig. 5A) was not sufficient to express the presumed change(s). A 40-min exposure (Fig. 5D) did not display the same acceleration in rate, probably because of the toxic effect exerted.

**Analysis of cell cycle parameters.** In a preliminary attempt to determine the contribution of each parameter to the decreased cell size (Fig. 4) and increased rate of division

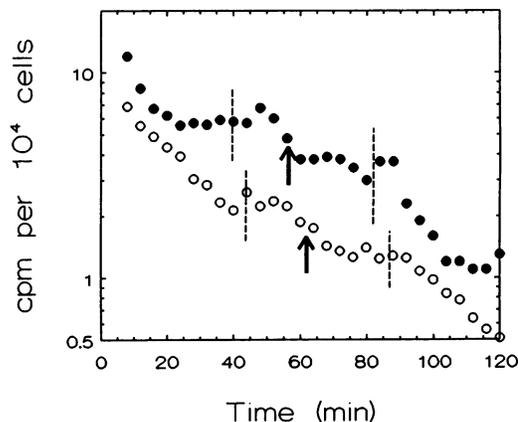


FIG. 6. Radioactivity (counts per minute) per ml in the eluates of baby-machine cultures as functions of time. ○, untreated control; ●, cultures treated with  $\alpha$ MG (1%). Arrows indicate the respective  $C + D$  times, which were taken in each case from the midpoint of the pulse-labeling interval to the midpoint of the drop between two successive plateaus. Vertical dashed lines indicate generations of elution.

(Fig. 5), a steadily growing culture was treated with  $\alpha$ MG for 25 min and pulse-labeled (4 min) with [<sup>3</sup>H]thymidine. The cells were then loaded on a filter in a baby machine and eluted with fresh medium without the analog (Fig. 6). The results, analyzed as described previously for the same strain (12, 13), indicate a reduction in the  $C + D$  period, from 62 to 56 min. This change is expected to cause a reduction in the size ( $M_b$ ) of an average baby cell of about 18%, if  $M_i$  is constant, because  $(25) M_b$  is calculated as  $\ln 2 \cdot M_i \cdot 2^{(C + D)/r/2}$ , and the doubling time (on the membrane) increased from 40 to 44 min.  $[M_b(\alpha\text{MG})/M_b(\text{control})] = 2^{56/44}/2^{62/40} = 0.82$ .] Since this theoretical reduction is less than the 25% observed (Fig. 4),  $M_i$  may also be reduced upon addition of  $\alpha$ MG, but the methodology is not sensitive enough to determine the magnitude of this change with accuracy.

The reduction in cell cycle parameters in the presence of  $\alpha$ MG without a significant change in growth rate differs from results of previous studies, in which elongation of the  $C + D$  period and reduction in  $M_i$  were exerted by low growth rates (2, 24, 32), mutations (2, 23, 28), or amino acid deprivation (6). We cannot as yet explain the reduction in these cell cycle parameters caused by  $\alpha$ MG. It could be due to varied effects such as changes in cell shape, pool sizes, differential gene expression, etc. The immediate transition between mass growth rates achieved by  $\alpha$ MG is currently being exploited to analyze the regulation of these major cell cycle parameters in the bacterial cell.

This work was supported in part by grant GM26429 from the National Institute of General Medical Sciences and by a grant from the U.S.-Israel Binational Science Foundation.

#### REFERENCES

1. Bremer, H., and P. P. Dennis. 1987. Modulation of chemical composition and other parameters of the cell by growth rate, p. 1527-1542. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
2. Churchward, G., E. Estiva, and H. Bremer. 1981. Growth rate-dependent control of chromosome replication initiation in *Escherichia coli*. *J. Bacteriol.* 145:1232-1238.

3. Cooper, S. 1991. Bacterial growth and division: biochemistry and regulation of prokaryotic and eukaryotic division cycles. Academic Press, Inc., San Diego, Calif.
4. D'Ari, R., and N. Nanninga (ed.). 1991. Cell shape and division in *Escherichia coli*. Fourth forum in microbiology. Res. Microbiol. 141:1-152.
5. Donachie, W. D. 1968. Relationship between cell size and time of initiation of DNA replication. Nature (London) 219:1077-1079.
6. Grossman, N., and E. Z. Ron. 1980. Initiation of deoxyribonucleic acid replication in *Escherichia coli* B: uncoupling from mass/deoxyribonucleic acid ratio. J. Bacteriol. 143:100-104.
7. Grover, N. B., A. Zaritsky, C. L. Woldringh, and R. F. Rosenberger. 1980. Dimensional rearrangement of rod-shaped bacteria following nutritional shift-up. I. Theory. J. Theor. Biol. 86:421-439.
8. Hansen, M. T., M. L. Pato, S. Molin, N. F. Fiil, and K. von Meyenburg. 1975. Simple downshift and resulting lack of correlation between ppGpp pool size and ribonucleic acid accumulation. J. Bacteriol. 122:585-591.
9. Helmstetter, C. E. 1967. Rate of DNA synthesis during the division cycle of *Escherichia coli*. J. Mol. Biol. 24:417-427.
10. Helmstetter, C. E. 1987. Timing of synthetic activities in the cell cycle, p. 1594-1605. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
11. Helmstetter, C. E., S. Cooper, O. Pierucci, and L. Revelas. 1968. The bacterial life sequence. Cold Spring Harbor Symp. Quant. Biol. 33:809-822.
12. Helmstetter, C. E., and A. C. Leonard. 1990. Involvement of cell shape in the replication and segregation of chromosomes in *Escherichia coli*. Res. Microbiol. 141:30-39.
13. Helmstetter, C. E., and O. Pierucci. 1976. DNA synthesis during the division cycle of three substrains of *Escherichia coli* B/r. J. Mol. Biol. 102:477-486.
14. Ingraham, J. L., O. Maaløe, and F. C. Neidhardt. 1983. Growth of the bacterial cell, p. 348-385. Sinauer Associates, Inc., Sunderland, Mass.
15. Johnsen, K., S. Molin, O. Karlstrom, and O. Maaløe. 1977. Control of protein synthesis in *Escherichia coli*: analysis of an energy source shift-down. J. Bacteriol. 131:18-29.
16. Kessler, D. P., and H. V. Rickenberg. 1963. The competitive inhibition of  $\alpha$ -methylglucoside uptake in *Escherichia coli*. Biochem. Biophys. Res. Commun. 10:482-487.
17. Kjeldgaard, N. O. 1961. The kinetics of RNA and protein formation in *Salmonella typhimurium* during the transition between different states of balanced growth. Biochim. Biophys. Acta 49:64-76.
18. Kjeldgaard, N. O., O. Maaløe, and M. Schaechter. 1958. The transition between different physiological states during balanced growth of *Salmonella typhimurium*. J. Gen. Microbiol. 19:607-616.
19. Maaløe, O., and M. E. Bentzon. 1985. Promoter collectives in bacteria, p. 48-61. In M. Schaechter, F. C. Neidhardt, J. L. Ingraham, and N. O. Kjeldgaard (ed.), The molecular biology of bacterial growth. Jones and Bartlett Publishers, Inc., Boston.
20. Maaløe, O., and N. O. Kjeldgaard. 1966. Control of macromolecular synthesis: a study of DNA, RNA, and protein synthesis in bacteria. W. A. Benjamin, Inc., New York.
21. Molin, S., K. von Meyenburg, O. Maaløe, M. T. Hansen, and M. L. Pato. 1977. Control of ribosome synthesis in *Escherichia coli*: analysis of an energy source shift-down. J. Bacteriol. 131:7-17.
22. Neidhardt, F. C., J. L. Ingraham, and M. Schaechter. 1990. Physiology of the bacterial cell: a molecular approach, p. 423. Sinauer Associates, Inc., Sutherland, Mass.
23. Petersen, S. K., and F. G. Hansen. 1991. A missense mutation in the *rpoC* gene affects chromosomal replication control in *Escherichia coli*. J. Bacteriol. 173:5200-5206.
24. Pierucci, O., M. Melzer, C. Querini, M. Rickert, and C. Krajewski. 1981. Comparison among patterns of macromolecular synthesis in *Escherichia coli* B/r at growth rates less and more than one doubling per hour at 37°C. J. Bacteriol. 148:684-696.
25. Pritchard, R. H., P. T. Barth, and J. Collins. 1968. Control of DNA synthesis in bacteria. Symp. Soc. Gen. Microbiol. 19:263-297.
26. Schaechter, M., O. Maaløe, and N. O. Kjeldgaard. 1958. Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. J. Gen. Microbiol. 19:592-606.
27. von Meyenburg, K. 1971. Transport-limited growth rates in a mutant of *Escherichia coli*. J. Bacteriol. 107:878-888.
28. von Meyenburg, K., and F. C. Hansen. 1987. Regulation of chromosome replication, p. 1555-1577. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
29. Woldringh, C. L., N. B. Grover, R. F. Rosenberger, and A. Zaritsky. 1980. Dimensional rearrangement of rod-shaped bacteria following nutritional shift-up. II. Experiments with *Escherichia coli* B/r. J. Theor. Biol. 86:441-454.
30. Zaritsky, A. 1975. On dimensional determination of rod-shaped bacteria. J. Theor. Biol. 54:243-248.
31. Zaritsky, A., C. L. Woldringh, N. B. Grover, J. Naaman, and R. F. Rosenberger. 1982. Growth and form in bacteria. Comments Mol. Cell. Biophys. 1:237-260.
32. Zaritsky, A., and S. Zabrovitz. 1981. DNA synthesis in *Escherichia coli* during a nutritional shift-up. Mol. Gen. Genet. 181:464-466.